

**BACTERIOLOGICAL AND MYCOLOGICAL PROFILE  
OF NEONATAL SEPTICEMIA IN A TERTIARY CARE  
HOSPITAL**

*Dissertation submitted to*

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**M.D. (MICROBIOLOGY)**

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## INTRODUCTION

Sepsis is a complex syndrome caused by an uncontrolled inflammatory response (SIR), of infectious origin, characterized by multiple manifestations which can result in dysfunction or failure of one or more organs and even death.<sup>64</sup>

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection in the first month of life.

Globally, WHO estimates 5 million neonatal deaths a year. 98% of these occur in developing countries. The commonest etiology is sepsis which constitutes more than one third of the causes.<sup>64</sup>

Neonatal septicemia is an important cause of morbidity and mortality among neonates in India, with an estimated incidence of approximately 4% in intramural live births.<sup>56</sup>

The spectrum of pathogens analyzed from hospital based data collected by National Neonatal Perinatal Database network from different centres in our country includes *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter spp* and *Acinetobacter*. Other pathogens contributing to neonatal sepsis includes *Staphylococcus aureus*, *Streptococcus viridians* and *Candida* species.<sup>47</sup>

Systemic infections due to pathogenic fungi are also assuming increasing importance in the neonatal intensive care units. The NICU

babies become colonized very early. About 10% of these babies get colonized in the first week of life and about 64% babies get colonized by 4 weeks of hospital stay<sup>24</sup>. Among pathogenic fungi responsible for NICU outbreaks, *C.albicans*, *C.parapsilosis* and *C.tropicalis* are notorious.

**Several factors contribute to the frequency and severity of neonatal infection and emphasize the importance of early accurate diagnosis and appropriate treatment.**

1. The presenting clinical manifestations in neonates with infection may mimic the features of other diseases, hence the diagnosis is delayed till the infection becomes severe.
2. The newborns especially preterm, have an immature immune system which is easily over come by invading organisms.
3. With the improvement of neonatal Intensive care, the survival rate of preterm and low birth weight newborn have increased. They form a high risk group for infection.
4. A variety of aetiological agents including bacteria, viruses, fungi, protozoa and mycoplasma can cause infection.
5. The laboratory tests available for diagnosis do not provide rapid result needed for early and quick appropriate treatment.
6. Infection caused by multidrug resistant organisms have increased, further complicating the management.

## **AIMS AND OBJECTIVES**

1. To identify the bacterial and fungal agents causing sepsis in the newborn.
2. To study the antimicrobial susceptibility pattern of the isolates with a view to formulate an empiric antibiotic regimen.
3. To detect emerging pattern of resistance in these organisms by standard methods.
4. To know the prevalence of Extended spectrum beta lactamases production in gram negative bacteria.



## **REVIEW OF LITERATURE**

### **Definition**

Neonatal septicemia is a disease of infants who are less than one month of age, are clinically ill and have positive blood culture<sup>76</sup>

Neonatal Sepsis can be divided into 2 subtypes depending upon whether the onset of symptoms is before 72 hrs of life (early onset) or later (late onset).

### **Early onset septicemia**

It is caused by organisms prevalent in genital tract or in the labour room and maternity operation theatre<sup>47</sup>.

### **Late onset septicemia**

It is acquired after delivery in the normal new born nursery neonatal intensive care unit or the community<sup>57</sup>. The onset of symptoms is usually delayed beyond 72hrs after birth. In most cases, symptoms appear by the end of first week or during second week of life.

### **Epidemiology**

The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-2003)<sup>56</sup> is 30 per 1000 live births. The database comprising 18 tertiary care neonatal units across India found that septicemia was the commonest clinical category with an incidence of 23 per 1000 live births, while the incidence of meningitis was reported to be 3 per 1000 live births. Among intramural births, *Klebsiella pneumoniae* was the most frequently isolated pathogen

(32.5%) followed by *Staphylococcus aureus* (13.6%). Among extramural neonates (referred from community/other hospitals) *Klebsiella pneumoniae* was again the commonest organism (27%) followed by *Staphylococcus aureus* (15%) and *Pseudomonas* (13%).

The incidence of sepsis varies from 7.1<sup>42</sup> to 38<sup>82</sup> per 1000 live births in Asia; 6.5 to 23/1000 live births in Africa, 3.5<sup>49</sup>- 8.9<sup>68</sup> per 1000 live births in South America and caribeans; 6 per 1000 live births in USA and Australia.<sup>64</sup>

### **Pathogenesis of Neonatal infection**

The fetus normally encounters no microorganisms during development and the newborn infant becomes harmlessly colonized by bacteria acquired from the birth canal and the environment. This is because of the barrier to infection provided by the placenta and membranes, the low pathogenicity of colonizing microorganisms and the relative competence of the baby's defense mechanisms. It is usually when one or other of these factors is altered that fetal or neonatal infection occurs.

### **Colonization of Healthy Infants**

The nature of colonizing organisms is determined by the pattern of flora in the birth canal and in the environment. Babies born at home are colonized by organisms, derived primarily from the mother. These organisms and those acquired from family members, tend to be

community derived antibiotic-sensitive organisms, of limited pathogenicity.

In the postnatal ward, the baby will acquire organisms from the ward environment, other babies and the clinical staff.

### **Colonization of intestinal tract**

The predominant intestinal organisms acquired by normal babies are members of the family Enterobacteriaceae (including *E coli*, *Klebsiella* spp and *Citrobacter* spp)

### **Colonization of respiratory tract:**

Colonization of the upper respiratory tract occurs rapidly and 90% of infants have positive pharyngeal cultures by the third day. Coagulase-negative staphylococci are the commonest, followed by *Streptococcus viridans* and *Staphylococcus aureus*.

### **Colonization of skin**

Skin colonization is very rapid, with the number of bacteria increasing 100 fold during the first week. Coagulase-negative staphylococci predominate but *Staphylococcus aureus* may be found in 65% of infants. A host of other organisms can be found, including yeasts and a range of saprophytic bacteria. The Umbilicus, perineum and axillae are the most heavily colonized.

### ***Colonization of infants on a neonatal intensive care unit:***

These babies are at greatest risk of becoming colonized by pathogens, which often show resistance to antibiotics. The pattern of bowel colonization is very different among sick preterm infants. Coagulase negative staphylococcus and antibiotic resistant gram negative organisms predominate.<sup>18,19</sup>

Skin colonization on the NICU is mainly by Coagulase negative staphylococcus, which can be isolated from over 90% of all positive cultures. *Staphylococcus epidermidis* accounts for 80% and *Staphylococcus haemolyticus* for almost all of the remainder.<sup>10</sup>

### **Balance between colonization and infection**

Most babies become colonized without becoming infected, but in others various host factors or the pathogenicity of the organisms result in tissue invasion and sepsis.

Intact skin and mucous membranes present an important barrier to microorganisms. Abrasions and cuts, mucosal injury, cannulae, catheters and endotracheal tubes all open the way for bacterial invasion.

Certain infective agents have an inherent ability to penetrate the placental barrier, often damaging the placenta. Ascent of the vaginal organisms into the uterine cavity prior to rupture of the membranes is rare. Once the membranes rupture, the risk increases progressively with time.

Vaginal delivery inevitably results in contamination and the beginning of colonization of the skin and the gut of the baby. Vaginal flora vary considerably from woman to woman and many cases of early onset neonatal sepsis result from the vaginal carriage of opportunistic pathogens.

In addition to the organisms acquired during birth all babies are subject to further microbiological contamination from the environment. People are the main source of such contamination.

Babies are less able to combat infection than the older children. This is further compromised by prematurity.

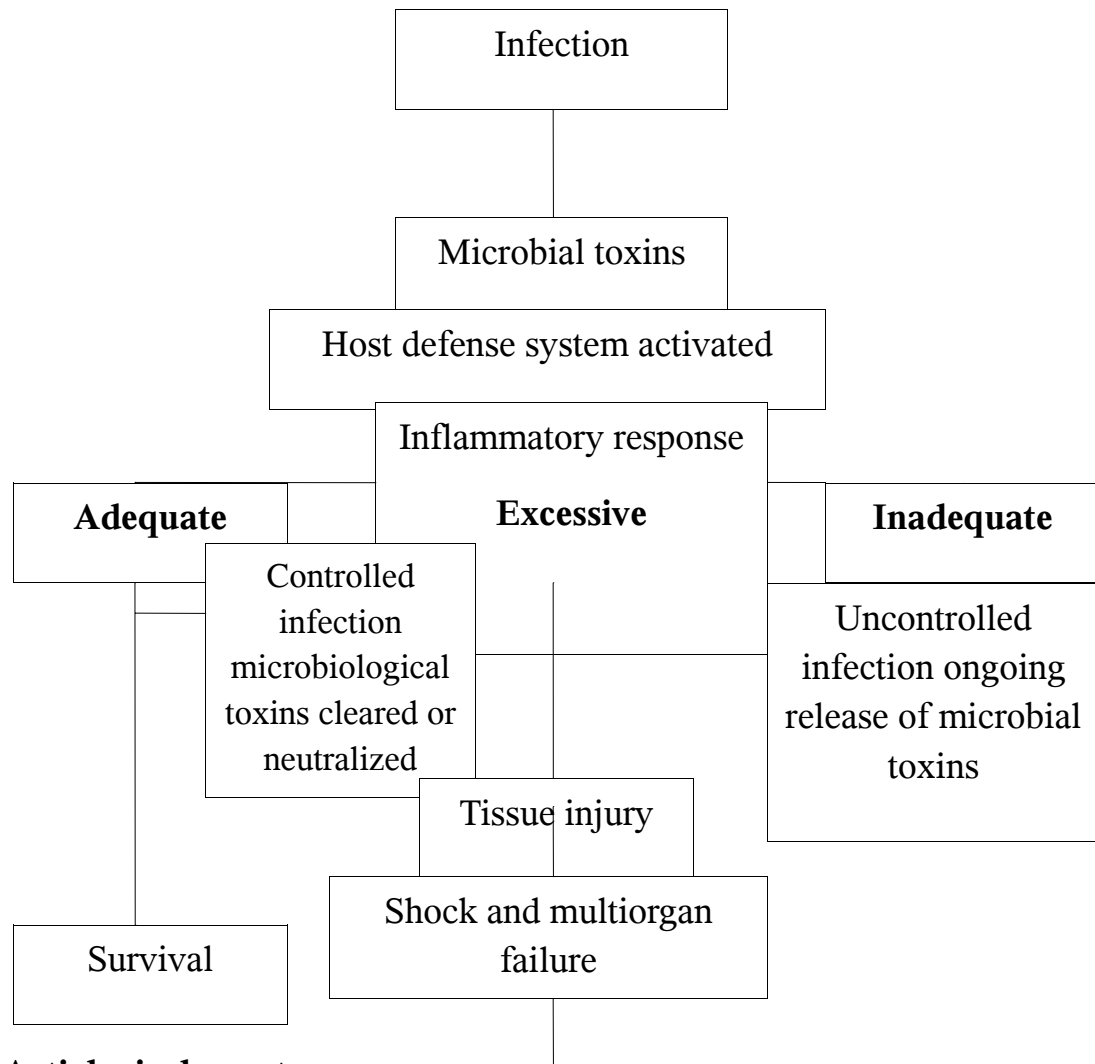
Competition between bacteria is a controlling influence on the level of colonization and risk of infection. The competitive inhibition of gram-negative organisms by *lactobacillus bifidus* in the gut of the breast fed infants.<sup>29</sup>

The various risk factors involved in neonatal sepsis are low birth weight<sup>85</sup>, prolonged rupture of membranes<sup>91</sup>, difficult delivery and instrumentation, birth asphyxia, difficult resuscitation<sup>35</sup>, ventilator therapy<sup>50</sup>, intravenous catheterization<sup>35</sup> and administration of parenteral nutrition<sup>86</sup>

## **PATHOGENESIS OF SEPSIS**

Infection begins when microorganisms circumvent or penetrate host barriers such as skin and mucosa. Depending on the infecting agents, virulence and immunocompetence of the patient, local host defenses may be overwhelmed, resulting in microbial invasion of the bloodstream. Toxic products released by the microbes in the circulation activate systemic host defenses, including plasma factors (Complement and clotting cascades) and cellular components (neutrophils, monocytes, macrophages and endothelial cells). In turn, activated cells produce potentially toxic mediators that augment the inflammatory response. This escalating immune response in concert with microbial toxins can lead to shock, multiple organ failure and death.<sup>11</sup>

## PATHOGENESIS OF SEPSIS



### Aetiological agents

#### Gram positive bacteria

*Staphylococcus aureus*

*albicans*

*Staphylococcus epidermidis*

*Streptococcus agalactiae*<sup>91</sup>

*Listeria monocytogenes*<sup>3</sup>

#### Death

#### Fungal agents

*Candida*

*Candida parapsilosis*<sup>66</sup>

*Candida tropicalis*<sup>17</sup>

*Malassezia furfur*<sup>7</sup>

*Malassezia pachydermatis*<sup>23</sup>

*Aspergillus fumigatus*<sup>46</sup>

### **Gram negative bacteria**

*Klebsiella pneumoniae*

*Escherichia coli*

*Pseudomonas aeruginosa*

*Hemophilus influenzae*

*Acinetobacter spp*

*Enterobacter aerogenes*<sup>58</sup>

### **Anaerobes**

Anaerobes have a role in causing early onset neonatal septicemia (Mitra S et al 1997).<sup>48</sup> Yohannan MD et al<sup>93</sup> 1992 stated that congenital pneumonia and early onset septicemia were caused by *Bacteroides fragilis*.

### **Gram positive organisms**

In the 1950s, phage group I *Staphylococcus aureus* was the most common bacterial agent that caused septicemia in neonatal units. Its unique invasive properties caused disseminated disease with widespread manifestations, including neonatal mastitis, furunculosis, septic arthritis, osteomyelitis and septicemia.

Methicillin-resistant organisms are an increasing problem in both neonatal and adult intensive care units and require treatment with



vancomycin. MRSA can be spread within Neonatal Intensive Care Unit in epidemic and endemic fashion. Infection control measures including identification of colonized infants by routine surveillance and cohorting and isolation of colonized infants may be required to prevent spread and persistence of the organism<sup>32</sup>.

Neonatal GBS infection is acquired in utero or during passage through the birth canal. because not all women are colonized with GBS, documented colonization with GBS is the strongest predictor of GBS Early onset sepsis. Heavy maternal colonisation [vaginal inoculum  $10^5$  colony forming units(cfu)/ml]with GBS is associated with a higher risk of invasive disease.

Among the *Coagulase negative staphylococcus*, *Staphylococcus epidermis* is the primary cause of NICU disease. *Coagulase negative staphylococci* universally colonize the skin of NICU patients. They are believed to cause bacteremia by first colonizing the surfaces of central catheters.<sup>32</sup>

### **Gram negative organisms**

Sepsis with Gram negative microorganisms is increasingly reported nowadays particularly in Asian countries<sup>12,33</sup>. The inadvertant use of broad spectrum antibiotics has led to the emergence of multi drug resistant gram negative bacteria<sup>37</sup>. *Klebsiella* species are of significant importance in this regard.<sup>69</sup>

*Escherichia coli* is another common gram negative bacteria that causes septicemia during neonatal period. Approximately 40% of *Escherichia coli* strains that cause septicemia possess K1 capsular antigen.<sup>92</sup>

In the newborns who receive broad spectrum antibiotics while in an environment that is potentially contaminated by bacteria from respirators or moist oxygen, the disease is most likely to be caused by *Pseudomonas* species<sup>92</sup>

*Acinetobacter* species is an important emerging nosocomial pathogen in neonatal septicemia<sup>25,88</sup>. Septicemia due to *Acinetobacter* spp are common in babies with predisposing factors like endotracheal intubation and artificial ventilation<sup>12</sup>. Other gram negative bacteria include *Proteus* species, *Enterobacter* species and *Citrobacter* species.

## **EXTENDED SPECTRUM BETA LACTAMASES**

In septicemic neonates, positivity of blood culture exhibits predominantly multi drug resistant gram negative rods among the isolates<sup>29</sup>

Multidrug resistant gram negative bacteria are associated with production of Extended spectrum beta lactamases. As these enzymes are active against narrow spectrum cephalosporins, Extended spectrum cephalosporins, ampicillin, the treatment becomes more problematic.<sup>62</sup>

Several outbreaks of infection caused by *Klebsiella pneumoniae* isolates that are simultaneously resistant to broad spectrum beta lactam antibiotics<sup>74</sup>. Studies carried out in various parts of India have reported prevalence of ESBL producing *Klebsiella* isolates.<sup>26</sup>

Jain et al observed ESBL production in 87.2% isolates of *Klebsiella* species, 65.3% isolates of *Escherichia coli* and 33.3% isolates of *Pseudomonas aeruginosa*.<sup>27</sup>

## CANDIDA INFECTIONS

Systemic candidiasis is a serious form of nosocomial infection in Very low birth weight infants. Recent data on late-onset sepsis from the NICHD Neonatal Research Network showed that 12% of cases of late-onset sepsis were caused by fungal species, primarily *Candida albicans* and *Candida parapsilosis*, and one-third of those infants died.

Risk factors for colonization and invasive diseases are similar. The administration of parenteral nutrition and intralipid infusions and the use of H2 blockers, systemic steroids and 3<sup>rd</sup> generation cephalosporins have been identified as independent risk factors for the development of invasive fungal infection<sup>30</sup>.

Apart from these factors low birth weight, pre maturity and intra venous catheterization makes neonates prone to candidemia<sup>20,80,78</sup>

There is some evidence showing a correlation between fungal colonization and invasive disease in very low birth weight babies<sup>6,40</sup>

## CLINICAL FEATURES

### Non-Specific features of sepsis are

1. Hypothermia or fever,
2. Lethargy, poor cry, refusal to suck.
3. Poor perfusion, prolonged capillary refill time
4. Hypotonia, absent neonatal reflexes
5. Bradycardia / tachycardia
6. Respiratory distress, apnoea and gasping respiration
7. Hypoglycemia / Hyperglycemia
8. Metabolic acidosis.

### Specific features, related to various systems are

CNS : Bulging anterior fontanelle, vacant stare, high-pitched cry, excess irritability, stupor / coma, seizures, neck retraction. Presence of these features should raise a clinical suspicion of meningitis.

Cardiovascular system: Hypotension, poor perfusion, shock.

GIT: Feed intolerance, Vomiting, Diarrhoea, Abdominal distension, Paralytic Ileus, Necrotizing enterocolitis (NEC)<sup>54</sup>

Hepatic : Hepatomegaly, direct hyperbilirubinemia (Jaundice)

Renal : Oliguria

Hematological : Bleeding, petechiae, purpura, splenomegaly

Skin Changes : Multiple pustules, abscess, sclerema, mottling, Umbilical redness and discharge.

## **LABORATORY INVESTIGATIONS**

### **Blood culture**

This is the definitive test, as the vast majority of neonatal infections are associated with bacteremia. Conventional blood culture takes 24-48 hrs to become positive. Prolonged incubation is necessary for some fastidious organisms like *Listeria monocytogenes*, *Haemophilus influenzae* and *Yeasts*.<sup>29</sup>

### **Sample Collection**

#### **Preparation of the site**

Careful skin preparation before collecting the blood sample is of paramount importance to reduce the risk of introducing contaminants into blood culture media.

Once a vein is selected, the skin over the venipuncture site is cleaned using 70% alcohol in a circular fashion. It is then allowed to dry. Starting in the centre of the circle, 2% tincture of iodine is applied in ever widening circles until the entire circle has been saturated with iodine. It is allowed to dry atleast for one minute.

The needle is inserted into the vein and blood is withdrawn. After the needle has been removed, the site is cleaned with 70% alcohol again, as many patients are sensitive to iodine.<sup>5</sup>

The blood is injected into the blood culture bottle under aseptic precautions.

### **Specimen volume:**

For infants and small children only 1 to 5ml of blood is drawn for culture. The blood is diluted between 1 in 5 and 1 in 10 in the culture medium to reduce concentration of natural antimicrobial constituents to a sub effective level.<sup>43</sup>

Smaller volumes of blood is sufficient since high levels of bacteremia (> 1000 cfu per ml of blood) are detected in some infants.<sup>5</sup>

### **Number of blood cultures**

To increase the chance of isolating pathogen, the specimens are collected at different time intervals. However a study found no significant difference in the yield between multiple blood cultures obtained simultaneously or those obtained at intervals.<sup>41</sup>

### **Sample processing**

Blood cultures are incubated for 24hrs at 37°C and observed for turbidity. Gram stain is done from the broth. Incubation periods are extended if slow growing organisms are suspected.<sup>84</sup> Apart from

conventional cultures, commercially developed blood culture systems and automated systems are also used.<sup>43</sup>

### **Buffy coat smear**

Gram staining of buffy layer of blood (after centrifugation) aid in the presumptive diagnosis of bacterial pathogens.

### **Indirect markers of neonatal sepsis:**

1. White cell counts – Leucopenia ( $<5000/\text{cu mm}$ ) or absolute neutropenia ( $<1500/\text{cu mm}$ )<sup>4</sup>
2. Band cell count  $>20\%$  and band count to total neutrophil ratio  $\geq 0.2$  is consistent with infection.
3. Micro ESR- A value of  $>15\text{mm}$  is considered as suggestive of infection.<sup>75</sup>
4. C-reactive protein  $>8\text{ }\mu\text{g}$  is abnormal in the neonate. Measurement of serial CRP concentrations in serum may be useful in the treatment of suspected neonatal sepsis.<sup>90</sup>
5. Presence of Dolhe bodie, toxic granules in blood smear is indicative of infection.
6. Platelet count, prothrombin time and partial thromboplastin time, fibrinogen level, arterial blood gas analysis and liver function test.
7. Biochemical analysis including blood glucose, urea, serum bilirubin, creatinine and electrolytes are also markers of underlying disease.

**Treatment**

Institution of prompt treatment is essential for ensuring optimum outcome of neonates with sepsis who often reach the health care facilities late and in a critical condition. Supportive care and antibiotics are the two equally important components of treatment.<sup>59</sup>

**Supportive treatment**

1. Warmth is provided to ensure consistently normal body temperature.
2. Intravenous line is started.
3. Normal saline 10 ml / kg is infused over 5-10 minutes, if perfusion is poor as evidenced by capillary refill time (CRT) of more than 3 seconds.
4. Glucose (10%) infusion 2 ml/kg stat.
5. Vitamin k 1 mg is given by intramuscular injection.
6. Nasal oxygen is started by hood or mask, if cyanosed or grunting
7. Gentle physical stimulation is given, if apnoeic
8. Bag and mask Ventilation with oxygen is provided if breathing is inadequate
9. Enteral feed is avoided in the very sick, maintenance fluids are given intravenously.
10. Exchange transfusion can be considered, if there is sclerema.



## **Specific treatment**

### **Antibacterial Treatment**

Antibiotic therapy should cover the common causative bacteria namely *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. A combination of ampicillin and gentamicin is recommended for treatment of sepsis and pneumonia. In cases of suspected meningitis, chloramphenicol should be added. Third generation Cephalosporins such as Cefotaxime and Amikacin are commonly used second line antibiotics.

### **Antifungal Treatment**

Immediately the diagnosis is made or even when there is a high level of suspicion of fungal infection in a sick infant, treatment with antifungals are started.

Amphotericin B is the mainstay of treatment for systemic fungal infections.<sup>65</sup> Because of the toxicity of Amphotericin, liposomal form of the drug, Ambisone is preferable nowadays. Flucytosine acts synergistically with Amphotericin and many authorities advocate their combined use in the initial treatment of fungal infection in the newborn.

Removal of central venous catheters and discontinuation of intravenous fat are other measures which can reduce the spread and severity of the infections.

**Immunotherapy**

1. Neutropenia is commonly observed in infants with proven bacterial infections. Granulocyte colony stimulating factor has been shown to resolve pre-eclampsia associated neutropenia and may thus decrease the rate of late onset sepsis in this population.<sup>63</sup>
2. Exchange transfusion.<sup>87</sup>
3. Fresh frozen plasma.<sup>81</sup>
4. Antilipid A monoclonal Antibodies.
5. Intravenous immunoglobulin administration.<sup>1</sup>
6. Anti TNF- $\alpha$
7. Interleukin-1 receptor antagonist

Other complications like acidosis, hypoglycemia are treated accordingly.

## **MATERIALS AND METHODS**

### **Place of study**

Institute of Microbiology  
Madras Medical College,  
Chennai-3

And

Department of Medical Newborn and  
Department of Microbiology,  
Institute of Child Health and Hospital for Children  
Egmore, Chennai-8

### **Period of Study**

Prospective study carried out over a period of one year from June  
2008-May 2009

### **Study Design**

Descriptive study.

### **Sample Specification**

Study population: 150 Newborn babies (<1month old) with clinical suspicion of sepsis were included in the prospective study.

A detailed prenatal and postnatal history with special emphasis on any predisposing factors for infection and thorough clinical assessment were carried out.

### **Inclusion criteria:**

Neonates with characteristics indicating probable sepsis:

- 1) Temperature > 99 degree F or 95 degree F.
- 2) Change in behavior - Abnormal cry or does not cry.
  - Not accepting feed.
  - Drowsy or unconscious.
  - Seizures
- 3) Septic focus on umbilicus or skin.
- 4) Diarrhoea
- 5) Rapid respiratory rate >60/mt.
- 6) Hospitalised or brought for consultation for other clinical illness and developing signs and symptoms of sepsis after hospitalization.

**Exclusion criteria**

- 1) No signs and symptoms indicating probable sepsis.
- 2) Prior antibiotic administration.

**Methodology****Sample Collection**

The skin over the Venipuncture site is cleaned with 70% alcohol and 2% tincture of iodine. About 0.5-1ml of blood was collected and directly inoculated into the Brain heart infusion broth and Robertson cooked meat broth with liquid paraffin overlay under aseptic precautions and incubated at 37 degree centigrade for 24hours.

**Sample processing**

After 24hrs of incubation, the cultures were examined for turbidity, lysis of red blood cells or clotting. From Brain heart infusion broth, a Gram smear was made and subcultures were done onto Mac conkey Agar, Blood Agar, Chocolate agar and Nutrient Agar and incubated at 37°C for 24hrs. Blood agar and Chocolate agar plates were incubated at 37°C in CO<sub>2</sub> environment (Candle jar). S/C were done onto two Sabouraud's Dextrose Agar slant, one incubated at 37°C and the other at 22°C.<sup>43</sup>

From Robertson cooked meat broth subculture was done onto 5% sheep blood agar with Kanamycin, Hemin and Vitamin K and was incubated under anaerobic environment for 72hours at 37° using polycarbonate jar with Gaspak.

All bottles were reincubated and checked for turbidity daily and subcultured whenever there were any signs of growth. A final subculture was made on the tenth day on to the above media<sup>43</sup>

### **Examination of subcultures**

The plates were examined after incubating for 18-24hrs. When any growth was seen, the colony morphology was noted. The colonies were identified by

1. Gram stain- to identify gram positive and gram negative organisms.
2. Hanging drop- to find out motile and non motile organisms.

3. Preliminary tests like Oxidase, Catalase were performed.
4. Members of the species were identified based on biochemical parameters using IMViC reaction and sugar fermentation medium.

## **COLONIAL MORPHOLOGY**

### ***Klebsiella pneumoniae***

#### **Nutrient agar**

Large , dome shaped mucoid colonies.

#### **Blood agar**

Large , grayish white, mucoid colonies.

#### **Mac Conkey agar**

Lactose fermenting mucoid colonies.

Species were identified with biochemical reactions

**BIOCHEMICAL REACTIONS**

Indole	Negative
Methyl red test	Negative
Voges Proskauer	Positive
Citrate	Utilised
Glucose	Acid with gas
Lactose	Acid with gas
Sucrose	Acid with gas
Maltose	Acid with gas
Mannitol	Acid with gas
Triple sugar iron	Acid / Acid with gas no H <sub>2</sub> S
Urease	Positive

***Escherichia coli:******Nutrient agar :***

*Colonies were 1-3 mm diameter, circular, low convex, smooth colonies with no pigmentation and odour.*

***Blood agar:***

*Greyish white colonies with haemolysis.*

***Mac Conkey agar:***

*Flat, lactose fermenting colonies.*

*Species were identified with biochemical reaction.*

**BIOCHEMICAL REACTIONS**

Indole	Positive
Methyl red test	Positive
Voges Proskauer	Negative
Citrate	Not utilised
Glucose	Acid with gas
Lactose	Acid with gas
Sucrose	Acid with gas
Maltose	Acid with gas
Mannitol	Acid with gas
Triple sugar iron	Acid / Acid with gas no H <sub>2</sub> S
Urease	Negative

***Pseudomonas aeruginosa******Nutrient agar:***

*Colonies were large, low convex with serrated margins, with bluish green pigmentation and earthy odour.*

***Blood agar:***

*Diffuse hemolysis present.*



**Mac Conkey agar:**

*Non lactose fermenting colonies with pigmentation .*

*Species were identified by biochemical reactions*

Indole	Negative
Methyl red test	Negative
Voges Proskauer	Negative
Citrate	Utilised
Glucose	Oxidatively utilised
Lactose	Oxidatively utilised
Mannitol	Not fermented
Xylose	Oxidatively utilised
Triple sugar iron	Alkali/ no change in the butt
O-F Test	Oxidatively utilised
Urease	Positive

**Acinetobacter species:****Blood agar:**

*Smooth, opaque , raised ,creamy colonies.*

**Mac Conkey agar**

*Non lactose fermenting colonies.*

Indole	Negative
Citrate	Utilised
Glucose	Not fermented
Triple sugar iron	Alkali/ no change in the butt
O-F Dextrose	Positive
Urease	Negative

**Staphylococcus aureus:****Nutrient agar:**

*Showed 1 to 3 mm diameter ,circular,smooth ,low convex,glistening, densely opaque colonies with golden yellow pigmentation.*

***Blood agar:***

*Colonies were surrounded by a narrow zone of beta hemolysis.*

***Mac Conkey agar:***

*Colonies were pink and small in size.*

*Slide Coagulase Test:positive*

*Tube coagulase test:positive*

***BIOCHEMICAL REACTIONS***

Indole	Negative
Methyl red	Positive
Voges Proskauer	Positive
Mannitol	Fermented
Urease	Positive

**Group B Streptococci (*Streptococcus agalactiae*)**

*Blood agar: colonies are 1-2mm diameter, grey, mucoid with  $\beta$  type of hemolysis*

**CAMP Test:**

*A sterile blood agar plate was taken. A single streak of *Staphylococcus aureus* ( $\beta$  hemolysin producing *Staphylococcus aureus*) was made in the centre of the plate. A streak of the  $\beta$  hemolytic *Streptococcus* was made perpendicular to the *Staphylococcal* streak, such that the two organisms were not touching each other. Known group A and Group B *Streptococcal* strains were inoculated in the same plate as negative and positive controls, respectively. The plate was incubated at 35°C in ambient air for 18-24 hours.*

*An area of increased hemolysis occurs where the  $\beta$  hemolysin secreted by *Staphylococcus* and the CAMP factor secreted by the Group B *Streptococcus* intersect.*

*Hippurate hydrolysis: To 0.4ml of 1% solution of sodium hippurate, a loopful of growth from blood agar was added and incubated at 37° C for two hours. After incubation 0.2ml of ninhydrin solution was added and incubated at 37°C for 10 minutes. The medium changes into purple colour due to hippurate hydrolysis.*

*Susceptibility to Bacitracin : Resistant.*

***Antimicrobial susceptibility tests:***

Antimicrobial susceptibility of the isolates were tested using Kirby Bauer disc diffusion technique as per CLSI guidelines<sup>16</sup>.

Medium used :MHA (Muller Hinton Blood agar plate was used for GBS isolate)

Inoculum : 0.5 McFarland turbidity, lawn Culture.

Incubation : 37°C ambient air, incubated for 16-18 hrs

**Preparation of inoculums:**

About 4-5 colonies of the same morphology were picked up with straight wire and inoculated in 5ml of peptone water, incubated at 37°C for 3-5 hrs to attain 0.5 Mc Farland's turbidity.<sup>5</sup>

A sterile cotton swab was dipped into it and pressed firmly against the wall of the test tube to remove excess broth from the swab.

Dried surface of Mueller Hinton Agar plate was swabbed in three directions approximately 60° each time to ensure an even and complete distribution of the inoculum over the entire plate

The anti microbial discs were dispensed on the agar plate and pressed down to ensure complete contact with agar surface. Disc were distributed evenly so that they were not closer than 24mm from centre to centre. Not more than 6 discs were placed in the plate.

After 16-18 hrs of incubation each plate was examined. The diameter of the zones of complete inhibition was measured including the diameter of the discs. Zones were measured to the nearest whole 'mm' using a ruler which was held on the back of the inverted petri plate.

**Control strains used were**

1. *Staphylococcus aureus* ATCC 25923
2. *Escherichia coli* ATCC25922
3. *Pseudomonas aeruginosa* ATCC 29823

***Screening of ESBL producing strains for Klebsiella pneumoniae***

Clinical and Laboratory Standards Institute has developed screening test for identifying the ESBL producing *Klebsiella* species.

According to CLSI guidelines, strains showing zones of inhibition of  $\leq 22\text{mm}$  for ceftazidime,  $\leq 27\text{mm}$  for cefotaxime were selected for conformational tests of ESBL. *Klebsiella pneumoniae* ATCC 700603 ESBL positive strain was used as control along with *Escherichia coli* ATCC 25922 as negative control.

***ESBL confirmatory test:***

**1. Double Disc Synergy test (DDST)<sup>30</sup>**

The isolated colonies were inoculated in peptone water at 37°C for 2-6 hrs. The turbidity was adjusted to 0.5 Mc Farlands standard and lawn culture was made on Muller Hinton agar using sterile swab.

Augmentin disc (20/10 µg) was placed in the centre of plate. Both side of Augmentin disc, a disc of cefotaxime (30 µg) and cetazidime (30 µg) were placed with centre to centre distance of 15mm to centrally placed disc. The plate was incubated at 37°C overnight, ESBL production was interpreted as the 3<sup>rd</sup> generation Cephalosporin disc inhibition was increased towards the Augmentin disc or if neither discs were inhibitory alone but bacterial growth was inhibited where the two antibiotics were diffused together.

## ***2. Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for ESBL<sup>89</sup>***

Antibiotic susceptibility testing was done on Muller Hinton Agar with 0.5 Mc Farlands standards of the organism. Lawn Culture of the organism was made and 3<sup>rd</sup> generation Cephalosporins Cefotaxime (30µg) disc and Cefotaxime with Clavulinic acid (30 µg + 10 µg ) disc was placed with 25mm apart. An increase of  $\geq 5$ mm in zone of inhibition for Cefotaxime with Clavulinic acid compared to Cefotaxime was confirmed as ESBL producers.

### ***Growth in Sabourauds Dextrose Agar is subjected for following tests:***

*The Sabourauds dextrose agar slopes were examined after 48hrs, 96 hrs, 5days,7days,14days and one month for the appearance of yeasty or mouldy growth.*

**GRAM STAIN**

Gram positive oval budding yeasts cells with presence or absence of pseudohyphae

**GERM TUBE TEST**

A loopful of creamy white yeast like colony from SDA was taken and it was inoculated in to 0.5ml of mammalian serum. It was incubated at 37 degree centigrade for one and a half to 2 hours. After incubation period, a loop full of this serum suspension was placed on a clean glass slide and covered with cover slip and focused under high power objective to see the characteristic germ tube formation .

**CHROMAGAR(HIMEDIA)**

A single colony from Sabourauds dextrose agar was taken and it was streaked on chromagar and incubated at 37°C for 48 hrs. After incubation period the plates were observed for characteristic coloured colonies of candida.

**CORNMEAL AGAR**

A single colony from Sabouraud's dextrose agar was inoculated on to plate of cornmeal agar containing 1% Tween 80 and trypan blue. Three parallel streaks were made about half an inch apart at a 45 degree angle to the culture medium. A sterile coverslip was placed over it and incubated at 30 degree centigrade for 48 hrs. After incubation the areas where the

cuts into the agar were made were examined for the presence of blastoconidia, arthroconidia, pseudohyphae, hyphae or chlamydoconidia.<sup>5</sup>

### **Carbohydrate fermentation test**

*A saline suspension of the yeast colonies was prepared. About 0.2ml of this suspension was inoculated on to the carbohydrate fermentation broth that contain Durhams tube and different sugars at a concentration of 2%. The different sugars used were glucose, lactose, sucrose, maltose respectively. The tubes were incubated at room temperature for 7-10 days. After incubation period, the tubes were observed for acid and gas production.*

### **Carbohydrate assimilation test**

Organisms from SDA were inoculated onto carbohydrate free medium either in nutrient agar or blood agar. A suspension of the yeast in saline or distilled water to a density equivalent to a McFarland No.4 standard was prepared. The saline suspension was swabbed on sterile yeast nitrogen base agar plate.<sup>5</sup>

The different carbohydrate disc used include glucose , lactose , sucrose, maltose, galactose, trehalose , raffinose. The disc were placed onto the surface of the agar approximately 30mm apart from each other. The plates were incubated at 30 degree centigrade for 24 to 48hrs.



After incubation, the plates were observed for the presence of a colour change around the carbohydrate containing discs or the presence of growth surrounding them<sup>5</sup>

## COLONY MORPHOLOGY

***Candida albicans:***

***Sabouraud's dextrose agar:*** colonies were cream coloured, pasty and smooth

***Gram stain:***

*Gram positive oval budding yeasts cells with or without pseudohyphae*

***Germ tube test:*** positive

***CHROMagar:*** colonies appear light green

**Cornmeal agar:**

Pseudohyphae in clusters with large, thick walled, terminal chlamydospores.

## Carbohydrate fermentation test

Glucose	Acid and gas
Maltose	Acid and gas
Sucrose	Not fermented
Lactose	Not fermented

**Carbohydrate assimilation test**

Assimilates glucose, maltose, sucrose, lactose, galactose , trehalose and xylose

***Candida tropicalis:*****Sabouraud's dextrose agar:**

Colonies were cream coloured, pasty, glistening to dull and smooth

**Gram stain:**

Gram positive oval budding yeasts cells with or without pseudohyphae

**Germ tube test :** negative

**CHROMagar:** blue coloured colonies with pink halo.

**Cornmeal agar:**

Short branched pseudohyphae with blastoconidia

**Carbohydrate fermentation test**

Glucose	Acid and gas
Maltose	Acid and gas
Sucrose	Acid and gas
Lactose	Not fermented

**Carbohydrate assimilation test**

Assimilates glucose, maltose , sucrose , galactose, trehalose and xylose.

## ANTIFUNGAL SUSCEPTIBILITY TESTS:

The antifungal susceptibility tests was done by two methods

- 1) DISC DIFFUSION METHOD
- 2) BROTH MICRODILUTION METHOD

### Inoculum preparation:

The inoculum suspension was prepared by picking five colonies, each of atleast 1mm in diameter, from 24 hour old cultures of *candida* species and suspending the material in 5ml of sterile saline. The suspension was then adjusted spectrophotometrically at 530nm to match the transmittance produced by 0.5 McFarland's barium sulphate standard. This procedure produces an inoculum size  $1 \times 10^6$  to  $5 \times 10^6$  cfu/ml. The same inoculum was used for both methods.<sup>60</sup>

The following standard strain was tested each time to ensure quality control :

*Candida albicans* ATCC 90028

### Disc diffusion method

It was performed on Muller Hinton agar plate supplemented with 2% glucose and 0.5 µg/ml methylene blue<sup>14</sup>

Antifungal susceptibility testing was carried out following the M 44-A. National Committee for Clinical Laboratory Standards (NCCLS) guidelines, using fluconazole and itraconazole antifungal discs.

## BROTH MICRODILUTION METHOD

This was done as per CLSI document M 27-A 2 for Yeasts, CLSI, Pennsylvania, USA 2002.<sup>16</sup>

The test was performed in a 96 well microtitre plate using standard RPMI1640 medium. MIC range of antifungal agents used were

- Amphotericin B : 0.03 – 16 micro gram/ml
- Fluconazole : 0.125 to 64 micro gram/ml

For fluconazole, Isolates with MIC 8 microgram /ml were considered susceptible, values between 16-32 microgram/ ml dose dependent susceptible and those 64 microgram/ml resistant. Due to the lack of defined breakpoints for Amphotericin B, isolates showing an MIC of  $1.0 \mu\text{g ml}^{-1}$  were taken as susceptible and those with MIC  $>1 \mu\text{g ml}^{-1}$  were considered as resistant<sup>67</sup>. The endpoint for Fluconazole was the lowest concentration in which prominent decrease in turbidity was observed and for Amphotericin B was defined as the lowest concentration in which an optically clear well was observed.<sup>70</sup>

## **RESULTS**

**TABLE – 1**

**AGE DISTRIBUTION OF CASES (n =150)**

55.33 % of Babies had Early onset sepsis and 44.67 % of babies had late onset sepsis. There is an almost equal incidence of early and late onset sepsis.

**TABLE - 2**

**SEX DISTRIBUTION OF CASES (n=150)**

Sepsis was more predominant in male babies (65.30%) than in female babies (34.70%).

**TABLE - 3**

**DISTRIBUTION OF CASES ACCORDING TO PERIOD OF GESTATION (n=150)**

The Number of Term babies with clinical signs of sepsis were 81 (54%) and Preterm were 69(46%).

**TABLE - 4**

**WEIGHT DISTRIBUTION OF CASES (n=150)**

According to National Neonatology forum Standards, Babies were considered as Low birth weight if the birth weight is <2.5Kg. In the Present study 120 (80%) of the neonates belong to Low birth weight category.

**TABLE - 5****PLACE OF DELIVERY : HOSPITAL VS HOME (n=150)**

92.7% of the cases had hospital delivery.

**TABLE - 6****BIRTH ORDER DISTRIBUTION OF CASES.**

Majority of the Babies with clinical evidence of sepsis were born to Primi gravida (48%).

**TABLE - 7****CORELATION BETWEEN PERIOD OF GESTATION AND ONSET OF SEPSIS**

Period of gestation	Early onset sepsis	Late onset sepsis
TERM (n=81)	38(46.9%)	43(53.1)
PRETERM(n=69)	45(65.2%)	24(34.8%)

P=0.02

Early onset sepsis was observed more in preterm neonates compared to term neonates.this is statistically significant.

**TABLE - 8****CORRELATION BETWEEN ONSET OF ILLNESS AND RISK FACTORS**

Low birth weight was the most common risk factor for both early and late onset sepsis.

LBW- Low birth weight, CRP- Child rearing practice, PROM- Premature rupture of membranes, PIH- Pregnancy induced hypertension.

**TABLE - 9**  
**ONSET OF ILLNESS AND CLINICAL MANIFESTATIONS**

Lethargy, Respiratory distress and seizures were observed to be the most common presenting manifestation in both early and late onset group of patients.

**TABLE - 10**  
**RESULTS OF BLOOD CULTURE (n=150)**

Blood culture was found to be positive in 62 (41.3%) cases.

**TABLE - 11**  
**ORGANISMS ISOLATED BY BLOOD CULTURE IN NEONATES  
WITH SEPSIS (n=62)**

Total culture positive cases were 62. Gram positive cocci were 23(37.1%), Gram negative bacilli were 35 (56.5%) candida was isolated in 4 (6.4%) cases.

**TABLE - 12**  
**ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE ORGANISMS**

Organism	Pen	Ampi	Oxa	Erythro	Cotri	Cef	Ak	Cip	Van
Staphylococcus aureus MSSA(n =10)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
MRSA (n=5)	0	0	0	2(40%)	3(60%)	0	5(100%)	3(60%)	5(100%)
Cons (n=4)	2 (50%)	2 (50%)	4 (100%)	3 (75%)	2 (50%)	4 (100%)	4 (100%)	3 (75%)	
Enterococci (n=2)	2 (100%)	2 (100%)		2 (100%)	2 (100%)	2 (100%)		2 (100%)	
Streptococcus agalactiae (n=2)	2 (100%)	2 (100%)		2 (100%)	2 (100%)	2 (100%)		2 (100%)	

All *Staphylococcus aureus* were sensitive to vancomycin.

**TABLE - 13**  
**ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE ORGANISMS**

Organism	AMPI	COTRI	CEF	AK	CEFTA ZIDIME	GENTA	CIP	IMI
Klebsiella pneumoniae (n =24)	6 (25%)	8 (33.3%)	10 (41.6%)	15 (62.5%)	13 (54.2%)	14 (58.3%)	20 (83.3%)	24 (100%)
Escherichia coli (n = 5)	3 (60%)	2 (40%)	3 (60%)	4 (80%)	3 (60%)	3 (60%)	4 (80%)	5 (100%)
Pseudomonas aeruginosa (n = 4)	1 (25%)		4 (100%)	4 (100%)	4 (100%)	1 (25%)	3 (75%)	4 (100%)
Acinetobacter SP(n=2)		2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)

All the isolates were sensitive to imipenem.



**TABLE - 14**  
**ESBL SCREENING TESTS FOR GRAM NEGATIVE ORGANISMS**

(Cefotaxime: zone of inhibition >27mm- sensitive, <27mm- resistant;  
 Ceftazidime, zone of inhibition >22mm- sensitive, < 22mm- resistant)

ORGANISM	SUSCEPTIBILITY TO 3 <sup>rd</sup> GCS		RESISTANT TO 3 <sup>rd</sup> GCS	
	No.of isolates	Percentage	No.of isolates	Percentage
<i>Klebsiella pneumonia</i> (n=24)	10	41.6%	14	58.3%
<i>Escherichia coli</i> (n=5)	3	60%	2	40%
<i>Pseudomonas aeruginosa</i> (n=4)	4	100%	–	–
<i>Acinetobacter</i> sp (n=2)	2	100%	–	–

14 isolates of *Klebsiella pneumoniae* and 2 isolates of *E.coli* were found resistant to third generation cephalosporins 3<sup>rd</sup> GCS – Third generation cephalosporins.

**TABLE - 15**  
**COMPARISON OF SCREENING TESTS WITH DDST AND PCDDT**

	Number of screened isolates for ESBL	DDST	PCDDT
<i>Klebsiella pneumoniae</i> (n=24)	14(58.3%)	8(33.3%)	9(37.5%)
<i>Escherichia coli</i> (n=5)	2(40%)	1(20%)	1(20%)

16 Isolates were selected and subjected for confirmatory tests. 10 Isolates were confirmed as ESBL Producers by PCDDT.

**TABLE - 16**  
**ANTIFUNGAL SUSCEPTIBILITY TESTS BY DISC DIFFUSION METHOD**

	FLUCONAZOLE		ITRACONAZOLE	
	SENSITIVE	RESISTANT	SENSITIVE	RESISTANT
C.albicans(n=3)	3(100%)	0	3(100%)	0
C.tropicalis(n=1)	0	1(100%)	0	1(100%)

*Candida tropicalis* was found to be resistant to both fluconazole and itraconazole.

**TABLE - 17**  
**ANTIFUNGAL SUSCEPTIBILITY TESTS BY MICROBROTH DILUTION METHOD**

	AMPHOTERICIN B		FLUCONAZOLE	
	SENSITIVE (MIC $\leq$ 1 $\mu$ g/ml)	RESISTANT (MIC > 1 $\mu$ g/ml)	SENSITIVE (MIC <64 $\mu$ g/ml)	RESISTANT (MIC >64 $\mu$ g/ml)
C.albicans (n=3)	3(100%)	0	3(100%)	0
C.tropicalis (n=1)	1(100%)	0	0	1(100%)

*Candida albicans* were susceptible to both Amphotericin B and Fluconazole.

**TABLE : 18**  
**ANALYSIS OF CLINICAL OUTCOME/MORTALITY IN NEONATES WITH SEPSIS (n=150)**

TOTAL NO. OF CASES	150
RECOVERED	134 (89.3%)
DEATH	16(10.7%)

10.7% mortality was observed among sepsis cases.

**TABLE : 19**  
**CORRELATION OF MORTALITY WITH TERM / PRETERM**  
**BABIES**

P=0.04

Mortality is observed to be high in preterm Babies (17.4%), Whereas in term Babies, it is less (4.9%). This is statistically significant.

**TABLE - 20**  
**ORGANISM ASSOCIATED WITH THE MORTALITY IN**  
**NEONATAL SEPSIS (n=16)**

Total no. of deaths	ORGANISM ISOLATED					
	Klebsiella pneumoniae	Pseudomonas aeruginosa	Staph. aureus	E.coli	Candida	No growth
16	6(37.5%)	2(12.5%)	2(12.5%)	2(12.5%)	2(12.5%)	2(12.5%)

There were a total of 16 deaths in the study, out of which 10 (62.5%) deaths were due to gram negative septicemia.

## DISCUSSION

The study was done at Institute of Microbiology, Madras Medical College and Institute of Child Health and Hospital for children. 150 newborn babies less than 30 days of age suspected to have sepsis were included in the study. Diagnosis of sepsis was arrived at on the basis of clinical examination.

Number of babies with early onset sepsis was 55.33% and late onset sepsis was 44.67%. This is similar to Meharban Singh et al<sup>47</sup> study where there is equal distribution of early and late onset cases.

According to age and sex distribution, neonatal infection was predominant in male babies (65.3%) as compared to female babies (34.7%). This correlated with the study conducted by H. William Teausch et al who found that<sup>92</sup> that neonatal septicemia was more common in male babies. S.S.Keshari et al<sup>36</sup> reported that the incidence of neonatal sepsis was predominant in male babies (63.01%) which correlated with the present study.

In the present study neonatal sepsis was more common in Primi Gravida (48%) followed by second Gravida (26%) which is in accordance with the finding that Primiparity is a risk factor for sepsis. Shah GS et al reported a higher incidence (72%) of neonatal sepsis in primi gravida.<sup>73</sup>

In this study the major risk factors for neonatal sepsis were low birth weight (80%) and prematurity (46%). Other risk factors found were

Rupture of membranes and delivery interval more than 12 hours (14%), bad child rearing practices (8%), pregnancy induced hypertension (6%) and cord around neck (2.6%).

Low birth weight and prematurity have been reported to be important risk factors for neonatal sepsis<sup>21,22,45 & 71</sup>.

Prematurity is a statistically significant risk factor for early onset sepsis in the present study.

Nathoo KJ et al 1990<sup>55</sup> reported that following were the risk factors for neonatal septicemia, poor antenatal care and prolonged rupture of membrane, whereas Tessin I et al 1990 reported that preterm delivery was a more important risk factor for both morbidity and mortality<sup>83</sup>.

The common clinical manifestations found in cases in this study were lethargy (48.7%), respiratory distress (42.1%), fever (30.7%), Jaundice (28%), seizure (24.6%), poor cry (23.3%), vomiting and diarrhoea (9.3%). Our study correlates with the study of Singhal PK et al 1992<sup>80</sup> who reported lethargy (81.4%), respiratory abnormalities (41.9%) and seizures (30.2%) as the most common clinical manifestations.

Jain N K et al also reported that common presentations found in neonatal sepsis were respiratory distress (42.6%), lethargy (40%), jaundice (30%), fever (28.3%) and poor feeding (26.7%).<sup>28</sup>

In the present study blood cultures were positive in 41.3% of cases. Aerobic gram-negative bacteremia was found in 56.5% cases. The predominant isolate was *Klebsiella pneumoniae* (38.7%). Others were *Escherichia-Coli* 8.1%, *Pseudomonas aeruginosa* 6.5% and *acinetobacter* species 3.2%. Gram-positive bacteremia was found in 37.1% cases. *Staphylococcus aureus* was the predominant isolate (24.2%), followed by *Coagulase negative staphylococci* (6.5%), *Enterococci* species (3.2%) and *Streptococcus agalactiae* (3.2%). No anaerobes were isolated in the present study.

Fungal isolates found were 6.4% .*Candida albicans* was isolated in 3cases and *Candida tropicalis* in 1case.

A study conducted by Kumhar GD et al showed blood culture positivity of 42.1%.The predominant isolates were *Klebsiella pneumoniae* 33.8% and *Staphylococcus aureus* 24.4%.The present study correlates well with these findings<sup>38</sup>.

Roy et al observed that the most frequent offender in neonatal sepsis were *Klebsiella* species(24.6%) followed by *Enterobacter* species (22.9%), *Coagulase negative staphylococci* (16.6%), *Staphylococcus aureus* (14%) and *Escherichia coli* (14%)<sup>70</sup>. Narang A, Kumar P et al reported *Staphylococcus aureus* (23%) as commonest isolate both in early and late onset sepsis<sup>53</sup>.

Out of 15 strains of *Staphylococcus aureus*, 10 (66.7%) showed sensitivity to oxacillin (1µg disc) by disc diffusion method; Methicillin Sensitive *Staphylococcus aureus* (MSSA) strains were also sensitive to Penicillin (100%), Ampicillin (100%), Cotrimoxazole (100%), Ciprofloxacin (100%) Amikacin (100%), and Erythromycin (100%).

The other 5 (33.3%) Strains of *Staphylococcus aureus* showed resistance to Oxacillin (1µg disc) and were sensitive to Vancomycin.

*Coagulase negative staphylococci* were sensitive to penicillin (50%), ampicillin (50%), cotrimoxazole (50%) and erythromycin (75%). all strains were sensitive to oxacillin.

*Group B Streptococci* were sensitive to Penicillin (100%), Ampicillin (100%), Cotrimoxazole (100%), Ciprofloxacin (100%) and Erythromycin (100%).

Among the gram-negative organisms, 58.3% of *Klebsiella pneumoniae* and 40% of *Escherichia coli* were found as ESBL producers by screening method.

By Phenotypic Confirmatory Disc Diffusion Test (PCDDT) 37.5% *Klebsiella pneumoniae* and 20% of *Escherichia coli* were confirmed as ESBL producers. All strains were sensitive to Imipenam. 80% isolates among the ESBL producers showed sensitivity to ciprofloxacin

A Bhattacharjee et al reported that out of 16 isolates of *Klebsiella pneumoniae*, 100% were screened positive for ESBL, whereas only 62.5% were confirmed as ESBL producers by PCDDT<sup>11</sup>.

CS Vinodkumar et al reported Extended spectrum beta lactamases (27%) among gram-negative isolates, *Klebsiella pneumonia* (48.6%) was the most frequent ESBL producing organism followed by *Escherichia coli* (16.8%) and *Pseudomonas* species (13.1%) which correlates with our study<sup>89</sup>.

In the present study *Pseudomonas aeruginosa* were sensitive to Amikacin (100%), ceftazidime (100%) ciprofloxacin (75%) and gentamycin (25%).

*Acinetobacter* species isolated from two cases showed 100% sensitivity to amikacin, cefotaxim, ciprofoxacin and cotrimoxazole.

*Candida albicans* was reported in 3(4.8%) cases and *Candida tropicalis* (1.6%) in one case in the present study. This is similar to Narain et al found that among neonatal candidemia, *Candida albicans* (54.4%) was the most common isolate from neonates followed by *Candida tropicalis* (23.3%) and *Candida krusei* (23.3%). Makhoul et al reported *Candida tropicalis* as the causative agent for 20% of episodes of neonatal fungemia<sup>44</sup>.

Scott K et al reported, *Candida albicans* blood stream infections were most common (57.9%), followed by *Candida parapsilosis*(33.7%),



*Candida tropicalis* (3.8%), *Candida lusitaniae* (2.3%), *Candida glabrata* (2%), and *Candida krusei* (0.2%) among the neonates.

Both *Candida albicans* and *Candida tropicalis* showed 100% Sensitivity to Amphotericin B. Fluconazole resistance was observed in *Candida tropicalis* isolate.

A study conducted by Law D et al reported that *Candida tropicalis* (48%) were resistant to fluconazole<sup>39</sup>.

Narain et al reported that all neonatal candidal isolates were sensitive to Amphotericin B(100%)<sup>51</sup>.

There were a total of 16 deaths in the present study. 12(75%) deaths in preterm babies and 4 (25%) deaths in term babies. The Mortality was higher in preterm neonates which was statistically significant

Out of 16 deaths, 62.5% deaths were due to gram negative organisms, 12.5% due to *Staphylococcus aureus* and 12.5% deaths by *candida*. Culture was negative in 12.5% of neonatal deaths. Pengsaa K et al 1996 found a mortality rate of 67.7% which is more than the mortality rate of this study<sup>61</sup>. Clemente Yago F et al 1992 reported a mortality rate 7.6% which is almost similar to our finding. Airede et al 1992(2) reported mortality rate of 27.3%<sup>13</sup>.

In the present study among the 16 deaths, 12.5% were due to ESBL producing *Klebsiella pneumoniae*.

Siu LK et al reported 25% mortality among the Neonatal septicemic cases due to ESBL producing *Klebsiella pneumoniae*<sup>79</sup>

The incidence of candidemia in my study was 12.5%. The mortality among the culture positive cases were 50%.. This is similar to the study by Jyotsna Agarwal et al who reported mortality rate was 52.6% in culture proven significant candidemia group<sup>34</sup>.

## SUMMARY & CONCLUSION

A prospective study was undertaken over a period of one year in 150 neonates to determine the bacterial and fungal isolates of neonatal sepsis.

Lethargy (48.7%) and Respiratory distress(42.15%) were the predominant presenting clinical findings. Preterm and low birth weight babies carried the highest risk of infection.

The spectrum of bacterial isolates among the neonatal sepsis were Gram positive cocci 37.1% and Gram negative bacilli 56.5% .

Fungi contributed to the remaining 6.4%.

The commonest organisms isolated were *Klebsiella pneumoniae* (38.7) and *Staphylococcus aureus* (24.2%).

The incidence of ESBL producing *Klebsiella pneumoniae* and *Escherichia coli* were 37.5% and 20% respectively. All strains were sensitive to Imipenam.

80% of Gram negative bacilli were sensitive to Amikacin, and Ciprofloxacin.

Among the *Staphylococcus aureus*, 66.7% were methycillin sensitive and 33.3% were methycillin resistant.

*Candida* was found in 6.4% of culture proven septicemia. The commonest being *Candida albicans* (75%). *Candida tropicalis* contributed to the remaining 25%.

All *candida* isolates were sensitive to Amphotericin B.

The overall mortality rate was 10.7%.

Presence of methycillin resistant *Staphylococcus aureus* and Extended spectrum  $\beta$  lactamase producing gram negative bacilli in the neonatal ward is of grave significance.

Continued surveillance of neonatal septicemia is mandatory due to the changing pattern of causative organisms and their antimicrobial susceptibility pattern.

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## PROFORMA

1. Reg number:
2. Name:
3. Age:
4. Address:
5. Birth order:
6. Risk factors:
  - a) MRO
  - b) Booked
  - c) Unbooked
7. Previous pregnancy:
  - a) Normal
  - b) Stillbirth
  - c) Neonatal death.
8. H/o Vaginal discharge:
  - a) Serous
  - b) Purulent
  - c) Mucus
  - d) Nil
9. Rupture of membrane and delivery interval(in hours
10. Mode of delivery
  - a) Hospital delivery-L.S.C.S or Normal delivery
  - b) Home delivery.
11. Nature of liquor:
  - a) Meconium stained
  - b) Foul smelling
  - c) Normal



12. Fever :
  - a. Antenatal:
  - b. Postnatal ( within 24 hours )
13. Present age of baby.
14. Gestational age of baby at birth:
15. Number of babies:
16. Birth weight: < 1000 gms: <1001- 1500gms: <1501- 2000gms: <2001 - 2500gms: <2501 - 3000gms: > 3001 gms:
17. General Condition:
  - \* Eyes: \*R.R: \*H.R:
  - \* Ear discharge:
  - \* Umbilicus:
  - \* Apnea spell:
  - \* Pallor :
  - \* Cyanosis:
  - \* Jaundice:
18. Respiratory distress:
19. Cardiovascular system:
20. Abdominal distention: Liver: spleen:
21. Anterior fontanel:
  1. Normal :
  2. Bulging:
  3. depressed;
22. Head circumference:
  1. Normal :
  2. Increased

23. Neonatal reflexes : Present / Absent
24. Any midline Swelling along with spine on the back :  
Present / Absent
25. Irritability : Present / Absent
26. Seizures : Present / Absent
27. Lethargy : Present / Absent
28. Poor Feeding : Present / Absent
29. Skin
30. Osteomyelitis :
31. Septic arthritis :

## INVESTIGATIONS

### Blood :

1. Complete Blood Count
2. Blood Sugar :  
Electrolytes :  
Blood            Urea  
                         Creatinine
3. Blood culture
4. Antibiotic / Antifungal susceptibility

### Urine

Albumin :  
Sugar  
Deposit

## TREATMENT :

Antibiotic / Antifungals administered  
Condition at the time of discharge

## **APPENDIX - I**

### **CULTURE MEDIA**

#### **BRAIN HEART INFUSION BROTH**

Sodium citrate 1gm

Sodium chloride 4gm

Sodium phosphate 5gm

Dextrose 10 gm

Peptone 10gm

#### **BRAIN HEART INFUSION**

Brain infusion broth      250ml

Heart infusion broth      750ml

Sodium polyanethol sulphonate      0.25gm

Obtain Ox brain and heart. Remove all fat from the heart. Cut into small pieces and grind. Add distilled water three times and keep at 4°C overnight.

From the brain, remove meninges fully and then, weigh. Add distilled water and mash by using hand. Keep in the cooler over night.

Next morning boil the brain and heart separately for 30 minutes. Then filtered through cotton layer. Measure each broth separately. Mix both infusions and the remaining ingredients. Dissolve well and adjust pH of the entire amount to 7.4 to 7.6.

Autoclave at 121° C for 15 minutes. Filter through filter paper and distribute in screw-capped bottles in 50 to 100ml amounts. Autoclave once more at 115° C for 10 minutes.

### **ROBERTSON'S COOKED MEAT MEDIUM**

Beef infusion broth

Minced and dried meat

Fat and connective tissue were removed from lean meat. Meat is mixed with 2 parts of water and refrigerated over night and any remaining fat is skimmed off. Boiled for 30 minutes. The mixture is filtered through 2 layers of gauze and the meat particles were dried. pH is adjusted to 7.4 to 7.6.

The dried meat particles were distributed in 12 X 100 mm test tubes to a height of 1.5 to 2.5 cm .The pH adjusted filtrate was then added to get 3-4 parts liquid per tube. The tubes were plugged and sterilized by autoclaving.

### **BLOODAGAR**

Sterile Defibrinated sheep blood -7 ml

Nutrient Agar (melted) -100ml

Nutrient agar was cooled to about 45-50 °C, 5-7ml of sterile defibrinated sheep blood was added. Mixed well and 15 ml of blood agar was poured in petri dishes.

**CHOCOLATE AGAR**

Sterile defibrinated blood - 10 ml

Nutrient Agar (melted) -100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

**MACCONKEY AGAR**

Peptone	-	2 gm
Sodium chloride	-	2 gm
Bile salt	-	0.5 gm
Lactose	-	1 gm
Agar	-	1.5 gm
Distilled water	-	100 ml

All the ingredients except lactose were dissolved in distilled water by heating. pH adjusted to 7.6. 1 ml of 1% neutral red solution was added to every 100 ml of medium with lactose and sterilized by autoclaving at 121°C for 15 minutes.

## MEDIA FOR BIOCHEMICAL IDENTIFICATION OF BACTERIA

Oxidase

Catalase

Indole

Triple Sugar Iron agar

Citrate

Urease

Methyl Red – Voges Proskauer

Nitrate reduction

Phenyl alanine deaminase test

Carbohydrate fermentation

Media

### 1. OXIDASE REAGENT

Tetra methyl P - phenylene diamine dihydro chloride -1% aqueous solution.

### 2. CATALASE TEST

3% Hydrogen peroxide

### 3. INDOLE REAGENT

**Kovac's reagent**

Para dimethyl amino benzaldehyde - 10gm

Iso amyl alcohol - 150ml

Hydrochloric acid - 80 ml

### 4. TRIPLE SUGAR IRON AGAR

Sodium chloride 0.5gm

Yeast extract 0.5gm

Peptone	2gm
Agar	1.5gm
Distilled water	100ml

Distilled by keeping in boiling water bath and the following ingredients were added.

Lactose	1.0gm
Sucrose	1.0gm
Dextrose	0.1 gm
Sodium thio sulphate	0.03gm
Ferrous sulphate	0.02 gm

pH adjusted to 7.6. Phenol red 0.0024 gm (2.4 ml of 1% solution) was added and distributed into test tubes in 4 ml quantities and autoclaved. The tubes were kept in a slanting position so that to get a deep butt and a short slant.

## 5. SIMMON'S CITRATE AGAR

Sodium Chloride	5.0g
Magnesium sulphate	0.2g
Ammonium dihydrogen phosphate	1.0g
Potassium dihydrogen phosphate	1.0g
Sodium citrate	5.0g.
Agar	20.0g

Bromothymol blue (1/500 aqueous solution) 40 ml

Distilled water 100 ml.

The ingredients were mixed and pH adjusted to 6.9. Sterilized by autoclaving and poured into tubes as slopes.

## 6. CHRISTENSEN'S UREA AGAR

### **Urea Solution**

Sodium Chloride	-5.0g
Dextrose	-1.0gm
Trypticase	-1.0gm
Mono potassium phosphate	-2.0gm
Urea	-20.0gm-
Distilled water	-100ml
Phenol red 1 % solution	-1.2 ml (in alcohol)

### **Urea agar base**

Agar	-1.5 gm
Distilled water	- 90 ml

The ingredients were dissolved in distilled water. pH adjusted to 6.8. Phenol red solution was added & sterilized by filtration. This is stock solution.

Agar was dissolved in distilled water and sterilized by autoclaving. Cooled to 45° C and 10 ml of urea solution was added, dispensed in 3-5 ml quantities and allowed to form a small butt and a long slant.



## 7. GLUCOSE PHOSPHATE BROTH (MR-VP medium)

Dipotassium phosphate	5.0gm
Glucose	5.0gm
Distilled water	100ml

The above ingredients were suspended in distilled water and heated slightly to dissolve them. Sterilized at 115° C for 15 minutes.

## 8. POTASSIUM NITRATE BROTH

Potassium nitrate (KNO <sub>3</sub> )	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

## 9. PHENYL ALANINE DEAMINASE TEST

Yeast Extract	3g	Dl-Phenylalanine	2 g
Disodium hydrogen phosphate	1g	Sodium Chloride	- 5 g
Agar	12g	Distilled water	- 1 lr

pH adjusted to 7.4, distributed in tubes and sterilized by autoclaving at 121° C for 15 minutes, allowed to solidify as long slopes.

## MEDIA FOR TESTING ANTIBIOTIC SENSITIVITY PATTERN Mueller - Hinton Agar

Beef extract	20.0gm
Acidicase peptone	7.5gm

Starch	1.5gm
Agar	17.0gm
Distilled water	1000ml

The ingredients were dissolved in one liter of distilled water, mixed thoroughly. Heated with frequent agitation and boiled for 1 minute. pH adjusted to 7.4  $\pm$  0.2. Sterilized by autoclaving and poured in plates.

## **APPENDIX - II**

### **Sabouraud's Dextrose Agar With Antibiotics**

Composition of Sabouraud's Dextrose Agar (Emmons Modification)

Dextrose	:	20 gm
Peptone	:	10 g
Agar	:	20 g
Distilled water	:	1000 ml
Final pH	:	6.9

The ingredients are dissolved by boiling. Gentamycin was dissolved in 10 ml of 95% alcohol and added to boiling medium. Autoclave at 121°C for 15 minutes, dispense in tubes and allow to cool in slanted position.

### **CORNMEAL AGAR**

Cornmeal	40g
Agar	15g
Water	1 litre

Boil the cornmeal in 1 litre of water for 60 min. Filter through muslin and add the agar. Steam to dissolve, dispense in required amounts and

autoclave at 115° C for 30min. allow to cool to 50°C and pour approximately 20 ml amounts into Petri dishes.

### **SUGAR FERMENTATION MEDIUM**

Peptone 15g

Andrade's indicator 10 ml

Sugar to be tested 20g

Water 1litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1litre of water and add 20g of the sugar; sugars to be tested generally include glucose ,sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3 consecutive days.

### **SUGAR ASSIMILATION AGAR**

Basal medium I

Yeast nitrogen base(Difco)

Agar

Water

Steam to dissolve and dispense in 10ml amounts in universal containers. Autoclave at 115 degree C for 15min.

## ABBREVIATIONS

ESBL	-	Extended Spectrum Beta Lactamases
DDST	-	Double Disk Synergy Test
PCDDT	-	Phenotypic Confirmatory Disc Diffusion Test
LBW	-	Low Birth Weight
NICU	-	Neonatal Intensive Care Unit
CRP	-	Child Rearing Practice
PROM	-	Premature Rupture Of Membranes
PIH	-	Pregnancy Induced Hypertension
MIC	-	Minimum Inhibitory Concentration
G1	-	Primi gravida
G2	-	Second gravida
G3	-	Third gravida
G4	-	Fourth gravida
G5	-	Fifth gravida

